RUO

INSTRUCTION FOR USE

HPV Panel PCR Kit

For Research Use Only

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1. INTENDED USE

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease. Furthermore, this test kit is not intended for the diagnosis of infectious diseases in animals.

The ***MarinaBiolab HPV Panel PCR Kit*** is a multiplex, qualitative Real-Time Polymerase Chain Reaction (qPCR) test intended for the simultaneous detection and identification of multiple pathogenic nucleic acids in research samples. The kit enables qPCR results in less than one hour. It is designed to detect gene sequences from the following organisms:

Targets	
HPV16	HPV35
HPV39	HPV58
HPV45	HPV56
HPV18	HPV33
HPV68	HPV31
HPV66	HPV51
HPV52	HPV59
Controls	
Human RNase P (IC)	
Bacillus atrophaeus (EC)	

2. PRINCIPLE of the PROCEDURE

DNA target regions are amplified using real-time PCR instruments, along with the specific primer and probe sets provided in the kit. During amplification, each probe binds to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase cleaves the probe, separating the reporter dye from the quencher and generating a fluorescent signal. With each cycle, more reporter dye molecules are released, resulting in an increase in fluorescence intensity. Fluorescence is measured at each cycle by the real-time PCR instrument. Probes labeled with distinct fluorophores are used to detect specific amplicons derived from both the target sequences and the internal control. The PCR instrument monitors the fluorescence signals in real time and interprets the data to provide a qualitative result for each target. A positive result for the presence of target DNA is indicated by the appearance of a real-time PCR amplification curve and a corresponding Cq (Quantification Cycle) value.

3. KIT COMPONENTS

The ***MarinaBiolab HPV Panel PCR Kit*** consists of four main components:

1. qPCR Enzyme and Buffer Mix (qPCR Master Mix)
2. Forward, Reverse and Probe Oligo Mix (HPV Oligo Mix 1-4)
3. A mixture of non-infectious DNA from artificial samples, including the targets listed in the table below (PC-HPV)
4. DNase/RNase-Free Water (NTC)

The components of the kit are provided in Table 1-2.

Table 1. Kit components.

Component	Description	Quantity x Volume
		100 rxn **MBLHPV016**
qPCR Master Mix	Ready-to-use mix for qPCR	2 x 1000 µL
HPV Oligo Mix 1-4	Primers and probes complementary to specific regions of the targets listed in the table above	4 x 250 µL
PC-HPV	A mixture of non-infectious DNA from artificial samples, including the targets listed in the table below	1 x 400 µL
NTC	DNase/RNase-Free Water	1 x 400 µL

Table 2. Oligo Mix target organisms and detection channels.

Vial Name	Target	Channel
HPV Oligo Mix 1	HPV16	**FAM/Green**
	HPV39	**HEX/VIC/JOE/Yellow**
	HPV45	**ROX/Texas Red/Orange**
	Human RNase P (IC)	**CY5/Red**
HPV Oligo Mix 2	HPV18	**FAM/Green**
	HPV68	**HEX/VIC/JOE/Yellow**
	HPV66	**ROX/Texas Red/Orange**
	Bacillus atrophaeus (EC)	**CY5/Red**
HPV Oligo Mix 3	HPV52	**FAM/Green**
	HPV35	**HEX/VIC/JOE/Yellow**
	HPV58	**ROX/Texas Red/Orange**
	HPV56	**CY5/Red**

HPV Oligo Mix 4	HPV33	**FAM/Green**
	HPV31	**HEX/VIC/JOE/Yellow**
	HPV51	**ROX/Texas Red/Orange**
	HPV59	**CY5/Red**

The oligonucleotide set targeting the human *RNase P* (Internal Control: IC) and *Bacillus atrophaeus* (External Control: EC) are used to monitor sampling, nucleic acid extraction, and inhibition of qPCR. The kit also contains negative and positive control templates to evaluate contamination and the qPCR reagent stability, respectively.

4. EQUIPMENT and MATERIALS REQUIRED but NOT PROVIDED

- 2-8°C Refrigerator
- ≤ -20°C Freezer
- ≤ -70°C Freezer (Optional)
- Vortex mixer
- Benchtop centrifuge with rotor for 1.5 mL tubes
- Benchtop mini centrifuge with rotor for PCR strips
- Benchtop plate centrifuge
- Biological Safety Cabinet (BSC)
- PCR cabinet for PCR Setup
- Adjustable Micropipettes: 1-10, 10-100, 100-1000 µL
- Sterile DNase/RNase free micropipettes tips - Compatible with the micropipettes
- Cold tube rack for microfuge tubes (1.5/2 mL) and for PCR tubes (0.1/0.2 mL)
- Disposable, powder-free, nitrile gloves
- Disposable (preferably) laboratory coat
- Surface decontaminants - Freshly diluted 10% bleach solution (0.5% NaClO)
- Applied Biosystems QuantStudio 5, 7, and 12K with Design & Analysis software and consumables
- Bio-Rad CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx/CFX384 Touch™/CFX Opus 384™ with Maestro software v1.1 and consumables
- Qiagen Rotor-Gene Q 5plex Platform with Rotor-Gene Q series software v2.1.0.9 and consumables
- Roche LightCycler 480 with software and consumables

5. WARNING and PRECAUTIONS

- The ***MarinaBiolab HPV Panel PCR Kit*** is intended for research use only and should be used by professionally trained, qualified personnel. All procedures should be performed in accordance with Good Laboratory Practices (GLP).
- Biological material used for nucleic acid extraction should be handled as potentially infectious. Appropriate safety precautions are recommended when handling biological material (e.g., do not pipet by mouth; wear disposable gloves; disinfect hands after completing the test).
- Biological material should be inactivated before disposal (e.g., autoclaving). Disposable items should be autoclaved or incinerated after use.
- In the event of a spill involving potentially infectious materials, the spill should be immediately absorbed with paper tissue, and the affected area should be disinfected using a suitable standard disinfectant or 70% alcohol. Materials used for cleaning spills, including gloves, should be inactivated before disposal (e.g., autoclaving).
- Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state, and local regulations.
- To avoid microbial contamination of reagents during aliquoting, it is recommended to use sterile, single-use pipettes and tips. Reagents that appear cloudy or show signs of microbial contamination should not be used.
- The kit should be stored away from nucleic acid sources and PCR amplicons to prevent contamination.
- Always check the expiration date on the kit. Do not use expired or improperly stored kits.
- Components in the kit should not be mixed with components from different lot numbers or from different manufacturers, even if they contain the same components.
- The kit components should be gently mixed before use by shaking.
- A common issue with PCR-based assays is false positive results caused by contamination from PCR amplicons. To minimize the risk of amplicon contamination:
    - Ensure separate work areas with dedicated apparatus are available for each stage of the procedure.
    - Do not open reaction tubes/plates post-amplification to avoid contamination with amplicons.
    - Discard used tubes/plates immediately in a biohazard container after completing the run.
    - Minimize handling of tubes/plates after testing.
    - Change gloves after handling used tubes/plates.

6. HANDLING, STORAGE, and STABILITY

- The ***MarinaBiolab HPV Panel PCR Kit*** is shipped on dry ice. If any component, except the qPCR Master Mix, is not frozen upon arrival or if the outer packaging has been compromised during shipment, please contact **MarinaBiolab** or the local distributor immediately.
- Upon arrival, all components should be stored between -25°C and -15°C.
- Repeated freezing and thawing of the kit components may reduce detection quality. The kit can withstand up to 15 freeze/thaw cycles without impacting performance.
- When stored under the specified conditions, the kit remains stable until the expiration date printed on the package. The expiration date is 12 months from the date of manufacture.
- All components must be thawed at ambient temperature for at least 30 minutes before use.
- It is recommended to keep all components on ice when preparing the assay mixes.
- The primer and probe mixes contain fluorophore-labeled probes and should be protected from direct sunlight and prolonged exposure to ambient light.
- Do not use expired or improperly stored components.

7. TEST PROCEDURE

7.1. Sample Preparation and Nucleic Acid Extraction

Samples intended for nucleic acid isolation must be collected using appropriate cell collection systems. The performance of the kit is highly dependent on both the quantity and quality of the extracted nucleic acid. Ensure that the extraction method used is compatible with real-time PCR technology.

If the laboratory's established standard protocol is used for nucleic acid isolation, it must be validated by the end user.

For frozen samples or previously extracted nucleic acid, thaw only the amount required for testing on the same day. Avoid multiple freeze/thaw cycles, as these can compromise nucleic acid integrity. For best results, use the nucleic acid immediately after thawing.

7.2. PCR Reaction Preparation and Processing

- Completely thaw all components at room temperature for at least 30 minutes prior to use.
- Once thawed, keep all components on ice throughout the entire testing procedure.
- Determine the number of reactions needed and prepare a PCR plate layout accordingly.
- The plate layout should include the following:
    - Reactions for each test sample and extraction negative control.
    - PCR control reactions:
        - Positive Control (provided in the kit)
        - Negative (No Template) Control (NTC) (provided in the kit)
        - No Template Addition Control (NRC)
- Vortex and briefly centrifuge all components before each use.
- Prepare a master mix by combining the required components for the total number of reactions plus an additional 10% to account for pipetting variability.

Table 3. Reaction set-up.

Reaction Mix Component	1X Reaction (µL) per well
qPCR Master Mix	5 µL
HPV Oligo Mix 1-4	2.5 µL
Template Nucleic Acid	2.5 µL
Total Reaction Volume	10 µL

- Add 5 µL of qPCR Master Mix and 2.5 µL of HPV Oligo Mix 1-4 to each PCR tube.
- Add 2.5 µL of the isolated sample to the corresponding tubes.
- The final reaction volume should be 10 µL.
- Close the tubes, centrifuge briefly, then place them into the real-time PCR instrument.
- Proceed with amplification using the PCR profile outlined below.

Table 4. Amplification profile.

Step	Number of Cycles	Temperature	Time	Data Collection
Initial Denaturation	1	95 °C	10 sec	**FAM/Green** **HEX/VIC/JOE/Yellow** **ROX/Texas Red/Orange** **CY5/Red**
Denaturation	40	95 °C	5 sec	
Annealing/Extension		55 °C	15 sec	

8. INTERPRETATION OF RESULTS

MarinaBiolab HPV Panel PCR Kit provides a qualitative result for the presence (Detected) or absence (Not Detected) of the target genes.

8.1. Calculation of Cq Values and Instrument-Specific Requirements

Configure the following instrument settings before evaluating the results.

Table 5. Instrument-specific settings.

Instrument	Threshold Level	Other Settings
CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx/ CFX384 Touch™/CFX Opus 384™ (Bio-Rad)	500 RFU	-
Rotor-Gene Q 5plex Platform (QIAGEN)	0.02 RFU	Dynamic Tube: Active Slope Correct: Active Outlier Removal: 0
QuantStudio™ 5, 7 and 12K (Applied Biosystems™)	Auto	-
Roche LightCycler 480 (Roche)	Auto	-

The shape of the amplification curves should be evaluated. If the instrument's software assigns a Cq value to a sample and the curve is sigmoidal, the Cq value can be used in the final assessment. *Non-sigmoidal curves should be recorded as negative.*

A result is considered positive if the Cq value is ≤35, or as determined by your laboratory's protocols.

8.2. Overall Validity of Detection

Table 6. Expected performance of controls.

Control Type	Used to Monitor	Signal	
		Target Channel	Internal/External Control Channel
Negative Control	Cross-contamination during extraction and reaction setup	-	-
No template addition	Reagent and/or environmental contamination	-	-
Positive Control	qPCR reaction setup and reagent integrity	+	+
Internal/External Control	To monitor the integrity of nucleic acid extraction and qPCR from each specimen	Not applicable	+

Before analyzing sample results, we recommend verifying the validity of the real-time PCR test. For each run, please confirm that the Positive and Negative controls performed as expected, based on the following criteria:

Table 7. Run validity/positive and negative control pass criteria.

Positive Control		Negative Control		Results	Recommendation
Target Channel	Internal/External Control Channel	Target Channel	Internal/External Control Channel		
+	+	-	-	VALID	Proceed with the interpretation of sample results.
Any of them is Negative		Not considered		INVALID	Contact the manufacturer, replenish the reagents, and repeat the reaction.
Not considered		Any of them is Positive		INVALID	Repeat the analysis, ensuring to follow the 'Warnings and Precautions' outlined in the IFU.

If any control fails to perform as described above, the run is considered invalid and must be repeated. If the issue persists, contact the manufacturer.

If all controls perform as expected, proceed with the interpretation of the results.

8.3. Interpretation of Unknown Specimen Results

The data generated by the instruments can be manually evaluated and reported using their software.

Table 8. Interpretation of unknown specimen results for DNA pathogens.

DNA Pathogens	Internal Control (*RNase P*)	External Control (*Bacillus atrophaeus*)	Results	Interpretation
Positive (+) (Cq<35)	Positive (+) (Cq<35)	Positive (+) (Cq<35)	Positive for Target	Target DNA is detected
Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Positive for Target	Target DNA is detected
Positive (+) (Cq<35)	Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Positive for Target	Target DNA is detected
Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Negative (-) (Cq≥35 or N/A)	Invalid	Repeat the test by re-extracting the sample. If the result remains invalid, consider collecting a new sample.
Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Positive (+) (Cq<35)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥35 or N/A)	Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥35 or N/A)	Positive (+) (Cq<35)	Negative (-) (Cq≥35 or N/A)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥35 or N/A)	Negative (-) (Cq≥35 or N/A)	Negative (-) (Cq≥35 or N/A)	Invalid	Repeat the test by re-extracting the sample. If the result remains invalid, consider collecting a new sample.

9. ASSAY LIMITATIONS

- The ***MarinaBiolab HPV Panel PCR Kit*** is intended for use only by professionally trained and qualified staff.
- A false negative result may occur if the specimen is improperly collected, transported, or handled. False negatives can also occur if amplification inhibitors are present in the specimen or if insufficient numbers of organisms are present.
- Spontaneous mutations within the target sequences may result in failure to detect the target. While the test design mitigates this risk, if target detection failure is anticipated, it is recommended to test the specimen with a different assay that targets other sequences in the genome.
- There is a risk of false positive results due to cross-contamination by target viruses and/or bacteria, their nucleic acids or amplified products, or from non-specific signals in the assay. Proper handling of consumables, as outlined in the Warnings and Precautions section, is crucial to minimize this risk.
- This assay is qualitative and does not provide a quantitative assessment of the detected organism's concentration.
- All instruments (e.g., pipettes, real-time PCR cyclers) must be calibrated according to the manufacturer's instructions.

10. PERFORMANCE CHARACTERISTICS

10.1. Analytical Sensitivity (Limit of Detection, LoD)

The limit of detection (LoD) was defined as the concentration at which the test produces a positive result more than 95% of the time. Serial dilutions of the strains were tested, and the initial tentative LoD was confirmed with twenty (20) replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, an additional twenty (20) replicates were performed at the next lower concentration until a detection rate of ≤95% was achieved.

For nucleic acid extraction, a simulated research matrix was spiked with strains and processed using the Automatic Nucleic Acids Extraction Instrument. Testing was carried out on the CFX96 Touch™ (Bio-Rad) Real-Time PCR system. The confirmed LoDs for the strains tested, along with the corresponding LoDs for the ***MarinaBiolab HPV Panel PCR Kit*** reportable targets, are presented in Table 9 below.

Table 9. Summary of LoD study results.

Analyte	Isolate ID/Source	LoD Concentration (copies/mL)	Detected/Total
HPV16	In-house	1.8E+02 copies/mL	**20/20** 100%
HPV39	In-house	1.5E+02 copies/mL	**20/20** 100%
HPV45	In-house	1.2E+02 copies/mL	**20/20** 100%
HPV18	In-house	1.1E+02 copies/mL	**20/20** 100%
HPV68	In-house	9.8E+01 copies/mL	**20/20** 100%
HPV66	In-house	8.8E+01 copies/mL	**20/20** 100%
HPV52	In-house	1.3E+02 copies/mL	**20/20** 100%
HPV35	In-house	1.1E+02 copies/mL	**20/20** 100%
HPV58	In-house	1.7E+02 copies/mL	**20/20** 100%
HPV56	In-house	1.5E+02 copies/mL	**20/20** 100%
HPV33	In-house	9.0E+01 copies/mL	**20/20** 100%
HPV31	In-house	1.1E+01 copies/mL	**20/20** 100%

HPV51	In-house	1.4E+01 copies/mL	**19/20** 95%
HPV59	In-house	9.7E+01 copies/mL	**19/20** 95%

10.2. Device Equivalence Study

A device equivalence study was conducted to assess the differences in results obtained using the kit across various instruments. For this purpose, the same LoD determination study was repeated using the Bio-Rad CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx/ CFX384 Touch™/CFX Opus 384™, Applied Biosystems QuantStudio 5, 7, and 12K, Qiagen Rotor-Gene Q 5plex Platform, and Roche LightCycler 480. Similar results were obtained at the 1x LoD concentration level of the targets in the device equivalence study across the different instruments.

10.3. Analytical Reactivity (Inclusivity)

10.3.1. In-Slico Analytical Reactivity

A BLAST search of the oligonucleotides was conducted on the genome sequences of HPV16, HPV39, HPV45, HPV18, HPV68, HPV66, HPV52, HPV35, HPV58, HPV56, HPV33, HPV31, HPV51, and HPV59 using the Primer-BLAST tool on the NCBI database.

The aggregated results of all in-silico analyses performed using the NCBI database are provided in the table below. The melting temperatures (Tm) of the oligonucleotide sequences with a 1-base mismatch remain higher than the annealing temperature specified in the PCR cycle parameters of the kit. Therefore, single base mismatches in the sequences are not expected to impact the inclusivity of the test.

Table 10. In-silico analysis results performed in the NCBI database.

Target	Primer	Total number of target sequences	Ratio of the sequences without mismatch	Ratio of the sequences with 1 base mismatch	Ratio of the sequences with 2 base mismatches	Ratio of the sequences with 3 base mismatches
HPV16	Sense Primer	4130	99.42%	0.58%	0.00%	0.00%
HPV16	Antisense Primer	4130	99.02%	0.98%	0.00%	0.00%
HPV16	Hydrolysis Probe	4100	99.89%	0.11%	0.00%	0.00%
HPV39	Sense Primer	50	100.00%	0.00%	0.00%	0.00%
HPV39	Antisense Primer	50	100.00%	0.00%	0.00%	0.00%
HPV39	Hydrolysis Probe	50	100.00%	0.00%	0.00%	0.00%
HPV45	Sense Primer	158	100.00%	0.00%	0.00%	0.00%
HPV45	Antisense Primer	158	100.00%	0.00%	0.00%	0.00%
HPV45	Hydrolysis Probe	154	100.00%	0.00%	0.00%	0.00%
HPV18	Sense Primer	171	99.68%	0.32%	0.00%	0.00%
HPV18	Antisense Primer	171	99.54%	0.46%	0.00%	0.00%

HPV18	Hydrolysis Probe	175	99.78%	0.22%	0.00%	0.00%
HPV68	Sense Primer	40	99.84%	0.16%	0.00%	0.00%
HPV68	Antisense Primer	40	99.82%	0.18%	0.00%	0.00%
HPV68	Hydrolysis Probe	39	99.91%	0.09%	0.00%	0.00%
HPV66	Sense Primer	65	99.82%	0.18%	0.00%	0.00%
HPV66	Antisense Primer	65	99.74%	0.26%	0.00%	0.00%
HPV66	Hydrolysis Probe	61	99.82%	0.18%	0.05%	0.00%
HPV52	Sense Primer	221	99.52%	0.48%	0.00%	0.00%
HPV52	Antisense Primer	221	99.67%	0.33%	0.00%	0.00%
HPV52	Hydrolysis Probe	230	99.83%	0.17%	0.00%	0.00%
HPV35	Sense Primer	926	100.00%	0.00%	0.00%	0.00%
HPV35	Antisense Primer	926	99.81%	0.19%	0.00%	0.00%
HPV35	Hydrolysis Probe	917	100.00%	0.00%	0.00%	0.00%
HPV58	Sense Primer	170	100.00%	0.00%	0.00%	0.00%
HPV58	Antisense Primer	170	100.00%	0.00%	0.00%	0.00%
HPV58	Hydrolysis Probe	172	100.00%	0.00%	0.00%	0.00%
HPV56	Sense Primer	72	100.00%	0.00%	0.00%	0.00%
HPV56	Antisense Primer	72	99.56%	0.44%	0.00%	0.00%
HPV56	Hydrolysis Probe	75	100.00%	0.00%	0.00%	0.00%
HPV33	Sense Primer	42	100.00%	0.00%	0.00%	0.00%
HPV33	Antisense Primer	42	100.00%	0.00%	0.00%	0.00%
HPV33	Hydrolysis Probe	45	100.00%	0.00%	0.00%	0.00%
HPV31	Sense Primer	2.141	100.00%	0.00%	0.00%	0.00%
HPV31	Antisense Primer	2.140	98.67%	1.33%	0.00%	0.00%
HPV31	Hydrolysis Probe	2.145	100.00%	0.00%	0.00%	0.00%
HPV51	Sense Primer	82	100.00%	0.00%	0.00%	0.00%
HPV51	Antisense Primer	82	98.47%	1,53%	0.00%	0.00%
HPV51	Hydrolysis Probe	87	98.59%	1.41%	0.00%	0.00%
HPV59	Sense Primer	78	100.00%	0.00%	0.00%	0.00%
HPV59	Antisense Primer	78	98.79%	1.21%	0.00%	0.00%
HPV59	Hydrolysis Probe	76	99.44%	0.56%	0.00%	0.00%

10.3.2. Wet-Test Analytical Reactivity

The analytical reactivity (inclusivity) of the ***MarinaBiolab HPV Panel PCR Kit*** was demonstrated using a comprehensive panel that represents the temporal, evolutionary, and geographic diversity of each target organism.

Each sample was tested in triplicate with the ***MarinaBiolab HPV Panel PCR Kit*** at an initial concentration 3-fold higher than the LoD determined for each analyte. In cases where the expected targets were not detected in one or more replicates, concentrations 3-fold higher were evaluated.

The individual strains and the concentrations at which positive test results were obtained for all three replicates are presented by target organisms in Table 11 below.

Table 11. Results of the wet inclusivity test.

Variant/Type/Subtype/Lineage/Genotype/Species	Isolate ID/Source	xLoD Detected
HPV16	In-house	1x
HPV39	In-house	1x
HPV45	In-house	1x
HPV18	In-house	1x
HPV68	In-house	1x
HPV66	In-house	1x
HPV52	In-house	1x
HPV35	In-house	1x
HPV58	In-house	1x
HPV56	In-house	1x
HPV33	In-house	1x
HPV31	In-house	1x
HPV51	In-house	1x
HPV59	In-house	1x

10.4. Analytical Specificity (Exclusivity)

10.4.1. In-Slico Analytical Specificity

Primers and probes designed for a target sequence may also bind to similar sequences if they closely match or differ by only a few base pairs from a non-targeted sequence. To ensure specificity to the target sequence, it is essential to screen the primers and probes against the reference database for the intended templates, as well as any databases that may contain potential contaminating templates.

Table 12. The results of On-Panel and Off-Panel organisms tested for cross-reactivity.

On-Panel/Off-Panel	Name of the organism	Cross Reactivity*		
		Forward	Probe	Reverse
On-Panel	HPV16	None	None	None
On-Panel	HPV39	None	None	None
On-Panel	HPV45	None	None	None
On-Panel	HPV18	None	None	None
On-Panel	HPV68	None	None	None
On-Panel	HPV66	None	None	None
On-Panel	HPV52	None	None	None
On-Panel	HPV35	None	None	None
On-Panel	HPV58	None	None	None
On-Panel	HPV56	None	None	None
On-Panel	HPV33	None	None	None
On-Panel	HPV31	None	None	None
On-Panel	HPV51	None	None	None
On-Panel	HPV59	None	None	None
Off-Panel	Herpes Simplex Virus 1	None	None	None
Off-Panel	Herpes Simplex Virus 2	None	None	None
Off-Panel	*Streptococcus agalactiae*	None	None	None
Off-Panel	*Treponema pallidum*	None	None	None
Off-Panel	*Gardnerella vaginalis*	None	None	None
Off-Panel	*Chlamydia trachomatis*	None	None	None
Off-Panel	*Neisseria gonorrhoeae*	None	None	None
Off-Panel	*Ureaplasma urealyticum*	None	None	None
Off-Panel	*Ureaplasma parvum*	None	None	None
Off-Panel	*Mycoplasma hominis*	None	None	None
Off-Panel	*Haemophilus ducreyi*	None	None	None
Off-Panel	*Trichomonas vaginalis*	None	None	None
Off-Panel	*Mycoplasma genitalium*	None	None	None
Off-Panel	*Acinetobacter calcoaceticus*	None	None	None
Off-Panel	*Acinetobacter baumannii*	None	None	None

Off-Panel	*Serratia marcescens*	None	None	None
Off-Panel	*Enterococcus faecalis*	None	None	None
Off-Panel	*Klebsiella aerogenes*	None	None	None
Off-Panel	*Klebsiella oxytoca*	None	None	None
Off-Panel	*Staphylococcus saprophyticus*	None	None	None
Off-Panel	*Staphylococcus aureus*	None	None	None
Off-Panel	*Klebsiella pneumoniae*	None	None	None
Off-Panel	*Proteus mirabilis*	None	None	None
Off-Panel	*Streptococcus agalactiae*	None	None	None
Off-Panel	*Proteus vulgaris*	None	None	None
Off-Panel	*Morganella morganii*	None	None	None
Off-Panel	*Citrobacter freundii*	None	None	None
Off-Panel	*Aerococcus urinae*	None	None	None
Off-Panel	*Candida glabrata*	None	None	None
Off-Panel	*Candida tropicalis*	None	None	None
Off-Panel	*Candida krusei*	None	None	None
Off-Panel	*Candida auris*	None	None	None
Off-Panel	*Candida parapsilosis*	None	None	None
Off-Panel	*Candida albicans*	None	None	None
Off-Panel	*Bacteroides fragilis*	None	None	None
Off-Panel	*Neisseria meningitidis*	None	None	None
Off-Panel	Sapovirus (I, II, IV, and V)	None	None	None
Off-Panel	Adenovirus F40/41	None	None	None
Off-Panel	Norovirus GI	None	None	None
Off-Panel	Norovirus GII	None	None	None
Off-Panel	Astrovirus	None	None	None
Off-Panel	Rotavirus A	None	None	None

* Homology should be <80% between the cross-reactivity microorganisms and the test primers/ probe(s).

10.4.2. Wet-Test Analytical Specificity

The potential for non-specific amplification by assays designed to detect analytes was evaluated by testing high concentrations of organisms or nucleic acids using the ***MarinaBiolab HPV Panel PCR Kit***. On-panel organisms were tested to assess potential intra-panel cross-reactivity, while off-panel organisms were tested to evaluate the specificity of the panel. Off-panel organisms included

normal flora, pathogens that may be present in specimens, and genetically related species to those detected by the ***MarinaBiolab HPV Panel PCR Kit***. The concentration of organisms tested (in triplicate) was at least 1.0E+06 CFU/mL for bacteria, fungi, and parasites, and at least 1.0E+05 units/mL for viruses. For certain organisms that were not available for laboratory testing, in silico analysis of the organism's whole genome sequences was used. The on-panel and off-panel organisms tested are listed in Table 13 and Table 14.

Table 13. On-Panel organisms tested for evaluation of ***MarinaBiolab HPV Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
HPV16	In-house	None
HPV39	In-house	None
HPV45	In-house	None
HPV18	In-house	None
HPV68	In-house	None
HPV66	In-house	None
HPV52	In-house	None
HPV35	In-house	None
HPV58	In-house	None
HPV56	In-house	None
HPV33	In-house	None
HPV31	In-house	None
HPV51	In-house	None
HPV59	In-house	None

Table 14. Off-Panel organisms were tested for evaluation of ***MarinaBiolab HPV Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Herpes Simplex Virus 1	ATCC VR-1778	None
Herpes Simplex Virus 2	Zeptometrix 0810217CF	None
Streptococcus agalactiae	ATCC 12386	None
Treponema pallidum	ATCC BAA-2642SD	None
Gardnerella vaginalis	ATCC 49145	None
Chlamydia trachomatis	Zeptometrix 0801775	None
Neisseria gonorrhoeae	ATCC 19424	None
Ureaplasma urealyticum	ATCC 27618	None

Ureaplasma parvum	ATCC 27815	None
Mycoplasma hominis	ATCC 27545-TTR	None
Haemophilus ducreyi	Zeptometrix 0801736DNA	None
Trichomonas vaginalis	ATCC 30001	None
Mycoplasma genitalium	ATCC 33530D	None
Acinetobacter calcoaceticus	ATCC 23055	None
Acinetobacter baumannii	ATCC 19606	None
Serratia marcescens	ATCC 29021	None
Enterococcus faecalis	Zeptometrix 0804216	None
Klebsiella aerogenes	ATCC 13048	None
Klebsiella oxytoca	ATCC 700324	None
Staphylococcus saprophyticus	Zeptometrix 0804014	None
Staphylococcus aureus	ATCC 10832	None
Klebsiella pneumoniae	NCTC 13465	None
Proteus mirabilis	Zeptometrix 0801544	None
Streptococcus agalactiae	ATCC 12386	None
Proteus vulgaris	ATCC 6380	None
Morganella morganii	Zeptometrix 0804010	None
Citrobacter freundii	Zeptometrix 0801563	None
Aerococcus urinae	ATCC 51268	None
Candida glabrata	ATCC 90030	None
Candida tropicalis	ATCC 750	None
Candida krusei	ATCC 2159	None
Candida auris	ATCC MYA-5003	None
Candida parapsilosis	ATCC 22019	None
Candida albicans	ATCC 10231	None
Bacteroides fragilis	ATCC 25285	None
Neisseria meningitidis	ATCC 13090	None
Sapovirus (I, II, IV, and V)	ATCC VR-3237SD	None
Adenovirus F40/41	Zeptometrix 0810084CF	None
Norovirus GI	ATCC VR-3234SD	None
Norovirus GII	ATCC VR-3235SD	None

Astrovirus	ATCC VR-1936	None
Rotavirus A	Zeptometrix 0810041CF	None

10.5. Interferences

The potential for endogenous or exogenous substances, which may be present in research samples or introduced during sample collection and handling, to interfere with the accurate detection of analytes was evaluated through select direct testing on the ***MarinaBiolab HPV Panel PCR Kit***. The findings were extrapolated from the interference evaluation of the kit.

Potentially interfering substances were evaluated using contrived samples spiked with the substance of interest. Results from samples containing the substance were compared to those from control samples without the substance. The substances tested included endogenous compounds that may be present in samples at normal or elevated levels (e.g., blood, mucus/mucin, human genomic DNA), various commensal or infectious microorganisms, medications, washes or topical applications, swabs and transport media used for sample collection, and substances employed to clean, decontaminate, or disinfect work areas. Each substance was added to contrived samples containing representative organisms at concentrations near (3x) the LoD. The concentration of each substance added to the samples was equal to or greater than the highest level expected in research samples, and each sample was tested in triplicate.

None of the substances tested were found to interfere with the ***MarinaBiolab HPV Panel PCR Kit***.

Table 15. Evaluation of potentially interfering substances on the ***MarinaBiolab HPV Panel PCR Kit***.

Substance Tested	Concentration Tested	Observed Interference
Endogenous Substances		
Human Blood	10% v/v	No Interference
Human Mucus	1 swab/mL sample	No Interference
Human Genomic DNA	20 ng/µL	No Interference
Human Urine	-	No Interference
Competitive Microorganisms		
HPV16	1.0E+05 CFU/mL	No Interference
HPV39	1.0E+05 CFU/mL	No Interference
HPV45	1.0E+05 CFU/mL	No Interference
HPV18	1.0E+05 CFU/mL	No Interference
HPV68	1.0E+05 CFU/mL	No Interference
HPV66	1.0E+05 CFU/mL	No Interference
HPV52	1.0E+05 CFU/mL	No Interference
HPV35	1.0E+05 CFU/mL	No Interference
HPV58	1.0E+05 CFU/mL	No Interference

HPV56	1.0E+05 CFU/mL	No Interference
HPV33	1.0E+05 CFU/mL	No Interference
HPV31	1.0E+05 CFU/mL	No Interference
HPV51	1.0E+05 CFU/mL	No Interference
HPV59	1.0E+05 CFU/mL	No Interference
Exogenous Substances		
K-Y Personal Lubricant Jelly	1% v/v	No Interference
Ortho Options Gynol II Extra Strength Vaginal Contraceptive Jelly	1% v/v	No Interference
Azithromycin	1.8 mg/mL	No Interference
Vagisil Creme Maximum Strength	1% w/v	No Interference
Aspirin	40 mg/mL	No Interference
K-Y Personal Lubricant Jelly	1% v/v	No Interference
Specimen Collection Materials		
Copan Liquid Amies Elution Swab (ESwab®)	N/A	No Interference

11. TROUBLESHOOTING

Problem	Cause	Solution
Target-specific and/or internal control (IC) signals were detected in the Negative Control well.	Contamination may arise from the environment, contamination of extraction and/or qPCR reagents, or well-to-well cross-contamination. The signal observed is not true target amplification, but rather background curves generated by the software of the qPCR instrument.	Repeat the qPCR using fresh reagents. Follow the general GLP guidelines in a PCR lab (e.g., decontaminate all surfaces and instruments with sodium hypochlorite or ethanol, and ensure filter tips are used and changed between samples). It is recommended to set up the qPCR reactions in a separate area, where no RNA/DNA is handled, and with equipment designated solely for pre-PCR activities. Ignore the Cq value of the No Template Control (NTC) if the amplification curve appears to be background noise rather than a true signal. If the issue persists, contact Technical Support.
No IC signal is detected, but a target-specific signal is observed in the sample wells.	A high copy number of target nucleic acid in the samples leads to preferential amplification of the target-specific nucleic acid.	No action is required. The result is considered positive.
The Positive Control did not meet the criteria for acceptable values specified by the kit, rendering the assay invalid.	The Positive Control was not stored under the recommended conditions. The kit has expired.	Check the kit label for the recommended storage conditions and expiration date. Replace the Positive Control. If necessary, use a new kit.
High Cq values were observed in the repeated samples.	The frozen samples were not mixed properly after thawing. Nucleic acids may be degraded.	Ensure frozen samples are thawed with mild agitation to guarantee thorough mixing. Make sure samples are stored correctly and are not subjected to multiple freeze-thaw cycles.
Target-specific and/or IC signals were detected after 35 cycles in the Positive Control.	Incorrect qPCR set-up or the kit reagents may have been compromised (e.g., improper storage or more than 15 freeze-thaw cycles).	Replace the control. If the problem persists, contact Technical Support.
No target-specific or IC signals were detected in the sample wells.	Sampling, extraction, or inhibition problem.	Dilute the nucleic acid isolate 1:10 and repeat the qPCR. If the diluted sample does not show a positive result in the IC channel, request a new sample and repeat the nucleic acid extraction. If necessary, repeat the nucleic acid extraction and the qPCR. If the issue persists, request a new sample, repeat the nucleic acid extraction and qPCR. If the problem continues, contact Technical Support.

12. EXPLANATION of SYMBOLS

Symbol	Title of Symbol	Symbol	Title of Symbol
RUO	Research Use Only		Use-by date
	Manufacturer	LOT	Batch code
CONTROL –	Negative control	NON STERILE	Non-sterile
CONTROL +	Positive control		Consult instructions for use or consult electronic instructions for use
CONTROL	Control		Caution
	Temperature limit	REF	Catalogue number
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Keep dry		Keep upright
Σ	Contains sufficient for <n> tests		Protect from heat and radioactive sources

Custom care and technical support

Tel: +1 510 579-5802

e-mail customer care: accounting@marinabiolab.com

e-mail Technical Support: rd@marinabiolab.com

MarinaBiolab LLC.

Address: 715 Discovery Blvd, suite 309 Cedar Park, TX 78613

For research use only (RUO)! Not for use in diagnostic procedures.